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TITLE: Changes in Ovarian Stromal Function in Premenopausal Women Undergoing
Chemotherapy for Breast Cancer

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See attached.

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ABSTRACT

The objective of this pilot study is to look for evidence of whether androgen levels are adversely affected by adjuvant chemotherapy for breast cancer and whether low androgen levels are correlated with the frequency and severity of fatigue, weight gain, psychological symptoms, vasomotor symptoms and libido. This pilot trial will use a longitudinal, descriptive design and will measure both subjective and objective measures of menopausal related phenomena in 20 premenopausal women receiving chemotherapy. Data collection will include blood draws and questionnaire completion at 4 time periods; baseline (before treatment), mid-treatment, immediate post-treatment and 6 months later. Questionnaires will include the Female Sexual Function Index, Greene Climacteric Scale, Profile of Mood States, cognitive and physical subscales of the Schwartz Fatigue Scale and a menses diary. Data analysis will involve descriptive statistics and plots of the hormone levels over time as well t-tests to examine changes in hormone levels. Correlational analysis will be done to look at the relationship of symptoms to various hormone levels. Final HSRRB and IRB approval received 4/05, recruitment began 6/1/05. If a connection between low levels of androgens and symptoms is found, androgen replacement may be a viable treatment option for breast cancer survivors.

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Statement of Work

Introduction

I would like to update you on the progress made with the Department of Defense on the protocol "Changes in Ovarian Stromal Function and Associated Symptoms in Premenopausal Women Undergoing Chemotherapy for Breast Cancer". Dr. Debra Barton was the original principal investigator on this protocol and received notification of this award on December 4, 2002. Dr. Barton subsequently changed positions and I officially became the principal investigator on this protocol 12/17/04. Since that time, emphasis has been placed on meeting the requirements of the HSRRB minutes of September 22, 2004, subsequent Mayo Clinic IRB approval for the protocol changes, obtaining a no cost extension, and ascertaining completion of needed paperwork so that the study could open. The study has just opened for accrual June 1, 2005.

Task 1: Work with a collaborative team to develop protocol of pilot study "Changes in ovarian stromal function in premenopausal women receiving chemotherapy for breast cancer" months 1 and 2

In the 2004 report, Dr. Barton reported that this task was completed. Since that time, changes have been made in the protocol per recommendations/considerations put forth by the HSRRB minutes of September 22, 2004. See appendix A (response to HSRRB minutes of September 22, 2004)

Task 2: Attend mentoring sessions and educational meetings, months 2 through 24

Dr. Barton attended regular mentoring sessions and educational meetings during the initial 24 months of this grant period.

I have participated in several mentoring experiences since becoming the principal investigator on this protocol. These include:

- Meetings with Dr. Loprinzi to discuss changes made in protocol per recommendations/consideration by HSRRB
- Meetings with Dr. Loprinzi to discuss recruitment issues
- Attendance at several educational meetings:
 - Monthly Oncology Nursing Society Meetings
 - Monthly Medical Oncology Society Meetings
 - Era of Hope 2005 Department of Defense Breast Cancer Research Program Meeting, Philadelphia, Pennsylvania - June 8 - 11, 2005
- Work with statistical team to enhance understanding of statistical approaches in which I have had limited experience. I have especially focused on learning more about logistic regression, confidence intervals, use of and communication of relative risk.
- Work with Dr. Lynn Hartmann and her research team on her Center of Excellence in Breast Cancer grant. I have attended bi-weekly team meetings, collaborated on writing of articles and strategic planning of ongoing activities. I have worked

closely with the team of study coordinators and statistical team in the collection, clean-up and analysis of data as well as in management of the budget.

Task 3: Get approval for pilot study, set up system to implement study, months 3 – 6

The pilot study protocol was initially approved by the Mayo Clinic IRB on November 26, 2002. Revisions made with DOD IRB were subsequently approved by Mayo Clinic IRB on 7/29/2003 and 7/6/2004. Responses to recommendations/considerations put forth by the HSRRB minutes of September 22, 2004 were approved by the DOD March 17, 2005. The changes were subsequently sent through Mayo Clinic IRB and received approval March 2005. With an original research end date of July 31, 2005 and award expiration of August 31, 2005, we submitted a request for a no-cost study extension. That approval was granted April, 2005.

Task 4: Accrue to study, months 7 -18

The study was officially opened upon completion of required paper work. The study was open for accrual June 1, 2005. We initially ran into difficulty with accrual in that the women who were identified by healthcare workers as eligible for this study, had decided to have their chemotherapy at their home institution. We have now worked with our laboratory to facilitate the patient blood draws at their home institution at the same time as their pre-treatment blood draws (when possible) and mailed back to Mayo Clinic for processing. To date we have accrued 2 women to this study. They have completed their baseline questionnaires and blood draws.

Task 5: Data entry and analysis, months 19-24

Accrual to study has just begun.

Task 6: Final analysis and report writing, month 24

Accrual to study had just begun.

Task 7: Strategize follow-up study and program of research based on pilot data, month 24

Key Accomplishments

- Addressed pre-review considerations included in the HSRRB minutes of September 22, 2004
- Approval by HSRRB March 17, 2005, pending completion of paperwork by Mayo's Institutional Official

- Approval of changes for HSRRB minutes of September 22, 2004 by Mayo Clinic IRB March, 2005
- Approval of a one year no-cost extension April 2005 (to extend research period to July 31, 2006)
- Mayo completion of required paper work May 25, 2005.
- Study opened for accrual June 1, 2005

Reportable Outcomes

- Poster Presentation at Era of Hope 2005 Department of Defense Breast Cancer Research Program Meeting, Philadelphia, Pennsylvania - June 8 - 11, 2005
- Poster Presentation accepted for Mayo Clinic Women's Cancer Program annual evening with researchers September 16, 2005
- Participated in a National Cancer Institute study section for SBIR Topics entitled: 211 - Developing Item Response Theory Software for Outcomes and Behavioral Measurement and 212 - Integrating Patient-Reported Outcomes in Clinical Oncology Practice and a Phase II proposal for The collection of Clinical Data Using Handheld Devices, April 1, 2005. This was done as a means to improve my knowledge of the grant review process.
- Reviewed manuscripts for *Journal of Clinical Oncology*, *Cancer*, *Psycho-Oncology*, *Mayo Proceedings* as a means to enhance my knowledge regarding critical writing skills and publications.

Conclusions

I have completed extensive self-study and mentorship with Dr. Loprinzi on the topic of endocrine changes resulting from chemotherapy use in women with newly diagnosed breast cancer. I have taken advantage of opportunities to build my knowledge in symptom management, statistical procedures, team approaches to research, NCI review process and article review processes.

The pilot study has been approved by appropriate DOD and Mayo Clinic review committees. Approval for a one year no-cost extension was received. To date, we have accrued two of the proposed 20 women to the study. Accrual and follow-up will be continued.

Appendix A: Response to HSRRB minutes of September 22, 2004

January 17, 2005

Commanding General
U.S. Army Medical Research and Materiel Command
ATTN: MCMR-ZB-QH (Dr. Beitins)
504 Scott Street
Fort Detrick, MD 21702-5012

RE: Department of Defense Clinical Research Nurse Award A-12155
"Changes in Ovarian Stromal Function in Premenopausal Women Undergoing
Chemotherapy for Breast Cancer"

Dear Dr. Beitins,

I have responded to the considerations from the Pre-review of the Protocol that were included in the HSRRB minutes of September 22, 2004 and transmitted to Dr. Barton by Ms. Kline on October 25, 2004. The responses are included in the attached document. I have also made the changes in the text of the protocol. A copy of the protocol with those changes are attached. Changes made in addition to those in the considerations include:

- SPSS will not be used for software. Thus, this was deleted from the proposal. Programs used by Cancer Center Statistics will be utilized. These include SAS and Mayo derived programs.
- References to Dr. Barton in the protocol were changed to Dr. Frost.
- We have a new Administrator for Human Research Protection. This was changed on the consent form.
- The inclusion criteria, in a committed relationship, was added in section 4.1.

I have also attached the following:

- Documentation regarding the change of principal investigator for the award
- Certification from Mayo that I have completed the training for the protection of human subjects
- A completed Conflict of Interest Form

Thank you for your assistance .

Sincerely,

Marlene H. Frost, RN, PhD, AOCN®

'Draft' Recommendations From the 22 September 2004 Army HSRRB Meeting for the Protocol Entitled, "Changes in Ovarian Stromal Function in Premenopausal Women Undergoing Chemotherapy for Breast Cancer," Submitted by Debra L. Barton, R.N., Ph.D., Mayo Clinic, Rochester, Minnesota, Proposal Log Number BC022090, Award Number DAMD17-03-1-0593, HSRRB Log Number A-12155 (HSRRB Vote: Conditional Approval)

CONSIDERATIONS FROM PRE-REVIEW OF THE PROTOCOL: The following were sent HSRRB members prior to the Board meeting. The Principal Investigator should consider addressing the following issues in the revised documents submitted to the Acting Chair, HSRRB.

COMMENT A. Sample size and power analysis. The Board suggested that each variable be listed in table format with the corresponding analysis (based on data from the literature) for statistical significance. The power analysis in the table (page 2 of the Attachment) does not include variables listed on page 2, section b., including androstenedione, sex hormone binding globulin, estrone, estradiol, DHEA-S, and FSH. It is stated that "These variables (other than the lab values) are descriptive factors to help clarify the generated hypotheses." There is no description of the methods to be used to analyze the data and correlate the findings to the data described in the table of research variables.

RESPONSE: The following chart and related literature justification will be added as section 7.33. This chart adds to the previous chart that was submitted.

VARIABLE	EXPECTED EFFECT SIZE	POWER	DATA ANALYSIS
Hormones		To detect changes over time	
Bioavailable Testosterone	20% - 30% decrease	80% power one sided t-test	-Descriptive statistics including means, standard deviation, range at each time point. -One sided t-test will be used to explore differences between baseline and the follow-up time points. Due to small sample size, no attempt will be made to conduct repeated measures statistics. -Visual plot of each woman's value plotted over time. -Descriptive statistics and visual plots will be used to explore trends in women who have the addition of tamoxifen and separately those who have the addition of a taxane.

			Due to small numbers, no statistical analyses to determine differences will be completed.
Androstendione	20% - 30% decrease	80% power one sided t-test	-Statistics described under bioavailable testosterone will be used.
Sex Hormone Binding Globulin	Unknown		-Statistics described under bioavailable testosterone will be used. -Pearson correlation to examine association between sex hormone binding globulin and bioavailable testosterone.
Estradiol	90% decrease	>80% power one sided t-test	-Statistics described under bioavailable testosterone will be used
Estrone	20% - 30% decrease	80% power one sided t-test	-Statistics described under bioavailable testosterone will be used
DHEA-S	20% - 30% decrease	80% power one sided t-test	- Statistics described under bioavailable testosterone will be used
FSH	40% or greater increase	>80% power one sided t-test	- Statistics described under bioavailable testosterone will be used
Symptoms*		Power to detect a statistically significant association	
Fatigue – Schwartz Cancer Fatigue Scale	.25 -.53	80% power for .53	-Pearson correlations between physical fatigue, cognitive fatigue and androgen levels
Libido – Female Sexual Function Index	.25 - .53	80% power for .53	-Pearson correlations between libido and androgen levels
Mood Changes – Profile of Mood States	.25 -.53	80% power for .53	-Pearson correlation between total mood disturbance and androgen levels
Vasomotor Symptoms – Greene Climateric Scale	.25-.53	80% power for .53	-Pearson correlations between vasomotor score and androgen levels

Menstruation, intensity of flow; spotting – Menstrual Cycle Diary	NA	NA	-Descriptive in terms of cessation and decreased flow.
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* Though statistical significance will not be evident unless the correlation effect is .53, for purposes of this exploratory study, we will consider .25 and higher correlations to warrant further study.

Premenopausal women with breast cancer who receive adjuvant chemotherapy are at increased risk of developing disrupted or repressed ovarian function. However, little is known about the effects of adjuvant therapy on testosterone. Thus, effect size is determined by level changes that occur with menopause.

Decreases in serum testosterone are similar to that of DHEA-S (Zumoff et al., 1995). DHEA-S peaks between 20 and 30 years of age. Between ages 21 and 40 there is a 50% decrease in DHEA-S. By age 60 DHEA-S levels are decreased 70% from peak levels. There are much smaller decreases after age 60 (Labrie et al., 2003). Thus, a 20% decrease would be similar to the decrease that occurs after menopause. As a precursor to testosterone, androstendione should also decrease 20%, if the mechanism of testosterone decrease is through this peripheral route. Another source notes that testosterone produced by the ovary decreases approximately 30% after menopause (Adashi, 1991).

Only 1% to 2% of testosterone circulates unbound in the plasma. The remainder is bound to sex-hormone-binding globulin (SHBG), a protein that is synthesized mainly in the liver. Factors that decrease binding of testosterone will increase the percentage of testosterone unbound, increasing its bioactivity (Copeland, 2000). A statistically significant decrease of SHBG will indicate this as a mechanism that has affected the availability of testosterone. A decrease that is not statistically significant, but greater than 20%, will warrant further investigation.

During menopause, estradiol levels decline to 20 to 25 pg/ml or less and estrone then becomes the principal circulating hormone (Copeland, 2000). If the woman's ovary function has been disrupted by chemotherapy, estradiol levels will decline. Estrone is formed in the peripheral tissues and is affected by the amount of adipose tissue (Copeland, 2000). In that androstendione is converted peripherally to estrone, we would anticipate a 20% or greater decrease consistent with the anticipated change in androstendione.

FSH concentrations increase sharply at the time of menopause, presenting a 5 to 6 fold increase. This hormone, also fluctuates during the menstrual cycle resulting in rises of 2 to 3 fold at mid-cycle from that at time of menstruation. Thus the rise greater than 3.5 fold will be indicative of changes that indicate beginning declines in ovarian function.

Estrogen produced by the ovary decreases below a critical level and slowly tapers to zero (Guyton, 1986).

Adashi EY. 1991 The climateric ovary: a viable endocrine organ. *Semin Reprod Endocrinol* 9:200-205.

Copeland LJ. 2000 Textbook of Gynecology. Saunders: Philadelphia.

Guyton AC. 1986. Textbook of medical physiology. Saunders: Philadelphia

Labrie F, Luu-the V, Labire C et al. 2003 Endocrine and intracrine sources of androgens in women: Inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. *Endocrine Reviews* 24:152-182.

Zumoff B, Strain GW, Miller LK, Rosner W. 1995 Twenty-four hour mean plasma testosterone concentration declines with age in normal premenopausal women. *J Clin Endocrinol Metab* 80:1429-1430.

COMMENT B. The need for a history and physical examination has been deleted from the protocol. Only the weight will be recorded. This limitation of the information will not allow for estimation of parameters valuable for interpretation of the collected data. Estimations of body mass index, level of stress, presence of symptoms besides those related to post-menopause, and the potential for self-selected methods to alleviate these and the menopausal symptoms may have a significant impact on the hormonal levels and/or the interpretation of the results.

RESPONSE:

- Height will be collected from chart data at the beginning of the study and weight will be collected at each time point. This will allow us to calculate body index. Descriptive statistics and visual plots will be used to explore trends in women who are underweight (below 18.5), normal (18.5 - 24.9), and overweight (25.0 - 29.9) according to their body mass index. In addition, histories and physical examinations will be routinely conducted prior to the patient starting chemotherapy and each time the patient is re-evaluated for another chemotherapy cycle. Since this is the case, the protocol did not wish to make another requirement for an additional examination. This has now been indicated in the protocol document (section 7.1). Having said this, we have found, in multiple symptoms control studies, that data collected by patient-completed questionnaires is much better than information obtained by histories. This information was added to section 7.34.
- Two stress questions will be added to the instrument section. These items have been used by the principal investigator in previous research and found to provide valuable information (Frost et al., 2000). Each item is rated on a 5 point Likert Scale with 1 = not stressful, 2 = slightly stressful, 3 = moderately stressful, 4 = very stressful, 5 = extremely stressful. The item stems are:

- Please rate the level of stress you have experienced during the past 4 weeks as a result of your health.
- Please rate how the stress you are experiencing now as a result of your health compares to previous or other current stressful situations in your life.

The stress questions will be analyzed using Pearson correlation to observe the association between perceived level of stress and other study variables. The items have been added as appendix VIII, the statistical approach reported in 7.34.

Frost M H, Vockley CW, Suman VJ, Greene MH, Zahasky K, Hartmann L. 2000
Perceived familial risk of cancer: Health concerns and psychosocial adjustment. J of Psychosocial Oncol 18(1):63-82.

- Questions will be added asking the patient at baseline and each successive time point to list any chronic illness, acute illnesses and symptoms related or unrelated to their breast cancer. They will also be asked to identify approaches they have used to deal with the illness or symptoms. These data will be reviewed for potential to impact hormone levels and/or interpret results. Any subgroup that exhibit a pattern different from the majority of women will be explored to see if chronic conditions, symptoms or interventions used differ between the groups. Items are added as appendix IX and analysis noted in section 7.34.

COMMENT C. Regarding the proposed correlations in the trend analysis (point 8 of the Recommendations) there is the expectation that the majority of women will experience abrupt and permanent cessation of menses secondary to chemotherapy. Review of the literature does not substantiate this view. A spectrum of responses based on the age, robustness of menstrual cyclicity, and the dose and type of chemotherapy could be encountered. Provisions for the statistical analysis of these potentially variable results should be considered.

RESPONSE:

This comment is correct, with regards to variability. Thus, the following will be done:

- All women will be on Adriamycin and Cytosan. Dosages will be recorded as well as the addition of tamoxifen or taxanes. Descriptive statistics and visual plots will be used to explore trends in women who have the addition of tamoxifen and separately those who have the addition of a taxane. Due to small numbers, no statistical analyses to determine differences will be completed. This description has been added to section 7.33.
- We will collect age and information about the regularity of menstrual periods in the year prior to starting chemotherapy, and explore androgen and estrogen differences based on these variables using analysis of variance models. A question about the regularity of menstruation in the last year was added to the demographic questionnaire as question 9 in Appendix VIII. This description has been added to section 7.34.

COMMENT D. Under the description of the management of adverse and unanticipated events, the responsibility of determining and reporting these events rests with the attending physician and the research participant. Since the adverse events could be limited to the blood draw, questionnaires, and loss of confidentiality, Dr. Barton should have the responsibility for the determination and reporting of the adverse or unanticipated events. Dr. Barton plans to report these events to the local IRB and USAMRMC and to the data monitoring and safety committee and data management group. Descriptions of these latter entities and their relationship to this protocol have not been provided in the protocol.

RESPONSE:

- The consent form has been changed to ask the patient to contact the principal investigator regarding any adverse effect. This was added under "What are the risks of this research study?"
- Data Monitoring and Safety Committee - This committee of the Mayo Clinic Cancer Center is responsible to serve Mayo Cancer Center studies. This committee monitors study accrual and all adverse effects.
- Data Management Group - Official members of the Data Monitoring and Safety Committee.

Appendix B: Statement of Work

Statement of Work

Task 1: Work with a collaborative team to develop protocol of pilot study "Changes in ovarian stromal function in premenopausal women receiving chemotherapy for breast cancer", months 1 and 2:

- a. Meet with co-investigators to discuss salient issues for pilot study.
- b. Draft protocol and send out for edits/input.
- c. Finalize protocol for submission to Protocol Review Committee.

Task 2: Start statistics classes, months 3 through 24:

- a. Enroll as non-degree student at the University of Minnesota.
- b. Confirm schedule for first class; Advanced Multiple Regression Analysis.
- c. Take one class per semester for a total of 3 advanced statistics courses.

Task 3: Attend mentoring sessions and educational meetings, months 2 through 24:

- a. Twice weekly meetings with Dr. Loprinzi.
- b. Weekly oncology core curriculum conference, breast committee and grand rounds attendance.
- c. Monthly cancer control meeting and monthly budget administration meeting.

Task 4: Get approval for pilot study, set up systems to implement study, months 3 – 6:

- a. Get protocol approved through Cancer Center Protocol Review Committee
- b. Obtain Institutional Review Board approval.
- c. Set up blood draws – research payment process
- d. Develop and copy questionnaire booklets
- e. Develop eligibility checklist and necessary data forms.

Task 5: Accrue to study, months 7 – 18:

- a. Work with nurse practitioners in breast clinic to identify eligible patients
- b. Educate, consent, follow patients through study

Task 6: Data entry and analysis, months 19-24:

- a. Set up data entry program
- b. Enter hematologic data and questionnaires
- c. Data cleaning and set up
- d. Preliminary analysis – look for problems, inconsistencies

Task 7: Final analysis and report writing, month 24

- a. Complete final data analysis
- b. Write draft of results for publication